PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
WO-02959		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/06227	14/06/2000	14/06/1999
International Patent Classification (IPC) or national classification and IPC C12N15/53		
Applicant DSM N.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
20/12/2000	04.09.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Friedrich, C Telephone No. +49 89 2399 7721

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/06227

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-26 as originally filed

Claims, No.:

1-27 as originally filed

Drawings, sheets:

1/6-6/6 as originally filed

Sequence listing part of the description, pages:

1-75, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 19-21, 23.

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 19-21, 23 are so unclear that no meaningful opinion could be formed (*specify*):
   see separate sheet

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 6-18, 22, 27
	No:	Claims 1-5, 24-26
Inventive step (IS)	Yes:	Claims 27
	No:	Claims 6-18, 22
Industrial applicability (IA)	Yes:	Claims 1-18, 22, 24-27
	No:	Claims

2. Citations and explanations
see separate sheet

Reference is made to the following documents:

D1: APARICIO, J.F. et al.,1999. The biosynthetic gene cluster for the 26-membered ring polyene macrolide pimaricin. A new polyketide synthase organization encoded by two subclusters separated by functionalization genes. Journal of Biological Chemistry 274:10133-10139.

D2: WO 95 01098 A (MONSANTO CO) 12 January 1995 (1995-01-12).

D3: US-A-5 672 497 (COX KAREN L ET AL) 30 September 1997 (1997-09-30).

D4: BRAUTASET, T. et al., 2000. Biosynthesis of the polyene antifungal antibiotic nystatin in Streptomyces noursei ATCC 11455: analysis of the gene cluster and deduction of the biosynthetic pathway. Chemistry and Biology 7:395-403.

Introduction

The present application concerns functionalization genes in the biosynthetic pathway of pimaricin. The claims refer to polynucleotide and amino acid sequences of three open reading frames (ORF1-3) within the polyketide synthase (PKS) cluster of Streptomyces natalensis (claims 1-5). They furthermore concern over expression (claims 6-8, and 15), inactivation (claims 9-11, and 16), and heterologous expression (claims 12-14, and 17) of said genes, generation of pimaricin, derivatives thereof, and other biomolecules (claims 18-23), and generation of recombinant protein (claims 24-27). No opinion has been established regarding subject-matter of claims 19-21 and 23, which refer to biomolecules. The application lacks unity under Rule 13.1, PCT, however, the international preliminary examination is performed in respect of the entire application. The applicant has not been invited to restrict the claims nor pay additional fees (Rule 68.1, PCT). Subject-matter concerning homologues and fragments of polypeptides and polynucleotides according to SEQ ID NO: 5-9 and their expression in cells (claims 1-5 and 24-26) is not considered novel under Art.33(1) and (2), PCT. Subject-matter concerning the cloning and use of known genes (claims 6-18, 22, and 24-26) is not considered inventive over D1 and D3 under Art.33(1) and (3).

The priority, as claimed, has been found valid. Thus D4, which is cited in the search report, has not been considered during examination of the present application.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Clarity of Claims, Art.6, PCT

Claims 19-21 and 23 refer to biomolecules without the provision of any technical features characterizing said biomolecules. In the light of the description the word biomolecule could mean e.g. a recombinant protein or a metabolite of the polyketide biosynthesis pathway. It could also mean any other molecule potentially produced by a cell. Since independent claims 19-21 and 23 do not contain the technical features essential to the definition of the invention, they do not comply with Art.6, PCT, and no opinion has been formed on novelty, inventive step and industrial applicability of said claims (see Art.34(4)(a)(ii), PCT).

Re Item IV

Lack of unity of invention

The present application concerns three different polynucleotides (SEQ ID NO: 5, 7, or 9) from the pimaricin biosynthesis gene cluster of S. natalensis and their use in the production of antibiotics (claims 1-27). Their biological activity as functionalization genes (cholesterol oxidase and p450-dependent monooxygenase) and their localization within the pimaricin biosynthetic gene cluster has already been disclosed in D1. Since the sequences are not structurally related there is no new special technical feature provided in the application which defines the contribution made by each individual invention over the prior art. The separate inventions, which are not so linked as to form a single general inventive concept (Rule 13.1 PCT), are:

1. SEQ ID NO: 5 and its use (claims 1-27 in part)
2. SEQ ID NO: 7 and its use (claims 1-27 in part)
3. SEQ ID NO: 9 and its use (claims 1-27 in part)

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Novelty, Art.33(1) and (2), PCT

1.1. Claims 1-5 refer to polynucleotide SEQ ID NO: 5, 7, or 9 and polypeptide SEQ ID NO: 6, 8, or 9 and homologues or fragments thereof, said homologues or fragments being defined based on sequence identity, sequence length and biologic activity (description p.8, l.27 to p.12, l.9 and examples 4 and 5). The biologic activities of SEQ ID NO:5 (ORF1), SEQ ID NO:7 (ORF2) and SEQ ID NO:9 (ORF3) were derived from comparison with sequences in public data bases (description, p.7, l.27-30). These sequences, based on which the function originally has been determined (e.g. the amino acid sequence of a 3-hydroxy-steroid-oxidase in D2, p.30-32 or cholesterol oxidases from other Streptomyces species described on page 7, line 31-32 of the present application), also fulfill the criteria for homologues or fragments of SEQ ID NO: 5-9 and thus appear to be novelty destroying for subject-matter of claims 1-5 according to Art.33 (1) and (2), PCT. Also vectors, cells, and recombinant expression of said sequences (claims 24-26) do not appear to be novel under Art.33(1)(2), PCT.

1.2. Overexpression (claims 6-8, 15, 18, and 22), inactivation (claims 9-11 and 16), and heterologous expression (claims 12-14, and 17) of polynucleotide SEQ ID NO: 5, 7, or 9, and oxidative modification with isolated polypeptides according to SEQ ID NO: 6, 8, or 9 (claim 27) have not been disclosed in the prior art and appear to be novel under Art.33(1)(2), PCT.

2. Inventive Step, Art.33(1) and (3), PCT

2.1. Claims 6-18, and 22 concern overexpression, inactivation, and heterologous expression, of isolated genes according to SEQ ID No. 5, 7, or 9 from the pimaricin biosynthetic pathway. D1 discloses the localization of said genes, namely ChoOx and two P-450 monooxygenases, next to pimS0 and pimS1 (page 10134, column 2, last paragraph and page 10135, Fig.2). The DNA sequence of pimS0 and pimS1 is also disclosed (AJ132221 and AJ132222). The function and roles of said genes in the pimaricin biosynthetic pathway were predicted based on sequence similarities and localization of the genes (D1, page 10134, column 2, 2nd paragraph). In the light of D1, which may be considered the closest prior art, the technical problem appears to be the

cloning of said genes and their use in the production of more or modified antibiotics. D3 discloses genes encoding enzymes required for the biosynthetic pathway of Tylosin, an antibiotic produced by Streptomyces fradiae. It further discloses the use of genes involved in the biosynthesis of Tylosin for the production of Tylosin at increased level (column 3, line 55 to column 4, line 15), the production of modified antibiotics (column 2, 3rd paragraph and column 19, lines 8-21), and mutant S. fradiae strains defective in Tylosin biosynthesis (column 10, last paragraph). Combination of the teaching of D1 and D3 gives strong incentive to clone the genes from S. natalensis flanking pimS0 and pimS1 and use them for recombinant expression. The actual cloning procedure and the generation of deficient or over expressing cell lines are considered well established methodologies widely used in the art before the relevant filing date of the present application. Thus the present invention amounts to not more than the application of known methods to allready localized genes, which can be readily performed by the skilled person in the art. Therefore the solution proposed in claims 6-18, and 22 of the present application does not appear to involve an inventive step under Art.33 (1) and (3), PCT.

2.2. The use of isolated polypeptide according to SEQ ID NO:6, 8, or 9 for the oxidative modification of a methyl group has not been disclosed or suggested by the prior art and thus appears to be inventive under Art.33 (1) and (3), PCT.

3. Industrial applicability, Art.33(1) and (4), PCT

Subject-matter where an opinion has been established (claims 1-18, 22, and 24-27, see also box III) appears to be industrially applicable under Art.33(1) and (4), PCT.

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